FOOD AND DRUG Research LABORATORIES, INC.

60 Evergreen Place
East Orange, New Jersey 07018
(201) 677-9500

ACCESSION NO. 106

BUTYLATED HYDROXYANISOLE

CAS REG. NO. MX 8003245

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Submitted to:

GRAS Review Branch (BF - 335)
Bureau of Foods
Food and Drug Administration
200 C Street, S. W.
Washington D. C., 20204

Att: Mr. Alan Spiher
Project Manager

Submitted by:

Harold Corey, Ph. D., Director
Science Information Group

Howard Feinman, Director
Biological Sciences

Date: June 29, 1973

Laboratory No. 1216

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BUTYLATED HYDROXYANISOLE

Summary - Toxicological Information

Extensive animal feeding studies over a wide range of intake levels indicate that BHA is without apparent toxic effect in test animals. The amounts administered ranged from 1 to 30,000 times the normal daily intake level for the human which is estimated to be on the order of 0.1 mg/kg (33). According to WHO (207a) the permissible daily dose of BHA is 0 - 0.5 mg/kg and the conditional permissible daily dose in 0.5 - 2.0 mg/kg.

For rats the oral LD_0, LD_{50} and LD_{100} values are 1,000, 2,900 and 4,500 mg/kg respectively, while for the mouse the LD_0, LD_{50} and LD_{100} values are 1,000, 1,250 and 3,000 mg/kg respectively (224).

In the rat and in the rabbit BHA is readily resorbed from the intestine and excreted in the urine after a short time, much of it coupled directly with glucuronic acid, while some smaller percentage appears coupled with sulfuric acid (148, 160). In the rat a small percentage of BHA is also demethylated so that the substances eliminated include the free phenol. In the dog only about half of the given dose passes across the intestinal wall and of this the major part is eliminated with sulfuric acid. A small percentage is also eliminated as demethylated and hydroxylated metabolic products (148).

In the human, the elimination products appear to be similar to those reported for the rat and rabbit except for the demethylation step which has not yet been demonstrated to occur. From single dose elimination studies of BHA in man it is known that about 50% of the antioxidant is excreted rapidly, appearing in the urine mainly as a glucuronide-coupled complex (34). The excretion rate then sharply decreases which to some degree is probably associated with the tissue storage and release rates of BHA and/or its metabolites. In one study using labeled BHA, 10% of the initial radioactivity remained within the system even after the eleventh day following ingestion of the antioxidant (100). However, tissue deposition per se appears not to be a significant factor in determining the kinetics of BHA metabolism, since there seems to be no tendency for the antioxidant to accumulate in tissues. Thus examination of muscle, liver, kidney and reserve fat of pigs and pullets receiving BHA daily in their diets over a period of several months showed no accumulation of the antioxidant in the tissues of these organs (142).

In one long term study weanling rats were fed BHA in diets for up to two years at levels as high as 0.5% of the fat in the diet (25 times the permissible amount of BHA in lard). No changes were observed in parameters of growth, food consumption, reproduction, mortality, organ weights and no organ tissue pathology was found (68). However, rats receiving BHA at a level of 0.1% of the diet registered an increased total serum cholesterol level, although higher doses of the antioxidant

produced no further elevations in the cholesterol (205). In this species, growth retardation was not evidenced, even at levels of BHA as high as 500 mg/kg given daily by stomach tubes (139).

While pathologic (68, 185, 224, 479), carcinogenic (186), or teratologic (94) symptoms associated with BHA intake were not observed in the animal studies recorded in the literature, the antioxidant was found to produce a number of abnormal physiological effects. Sub-acute toxicity experiments with animals receiving 1/5 th and 1/10 th their respective LD_{50} levels of BHA in the diet resulted in a decrease in the growth rates (224, 139) and a reduction in blood enzyme activities specifically that of catalase, peroxidase and cholinesterase (224). Other blood indexes such as glycogen, phospholipids, fat and total solids remained unchanged (227).

Liver weight and liver enzyme activities in rats were not affected by BHA when fed at levels below 0.25% of the diet while at the 0.5% level enzyme activity was found to have increased somewhat without a corresponding change in the liver weights which remained within the normal range (97). At the 500 mg/kg intake level hepatomegaly resulted, however, this was not accompanied by fatty changes (139). The absence of any hepatotoxic action due to BHA suggests that the enlargement of the liver is rather the result of hyper-functional activity (150). In contrast with BHT, the BHA induced hepatomegaly is not a result of increases in drug metabolizing enzymes (157, 158)

and specifically BHA was without effect on the glucose-6-phosphatase activity in the liver (138).

Other enzyme systems monitored were also found not to be interfered with by BHA when fed to groups of rats for up to three months. In these animals no changes were noted in enterokinase and alkaline phosphatase activities in the mucosa of the small intestine, nor in the activity of the pancreatic amylase and lipase (228). This "no effect" observation is in contrast to the findings reported above (97) in which increased liver enzyme activity associated with BHA intake was observed and to the report of increased liver succinate dehydrogenase activity in rats receiving 1% BHA in the fat of their diet over a period of eight weeks (407).

No adverse effects were noted during the course of long term feeding studies with dogs receiving BHA at levels as high as 1,000 times the normal human intake level (200 times the permissible level). There were no unusual changes in urinary protein and sugar, nor in hematologic values. Normal growth was maintained in these animals, and there was no evidence of BHA storage in fat, brain, liver and kidney tissue (185). Only in animals receiving doses as high as 2,500 times the normal human intake level (500 times the permissible level) was evidence of liver injury found to have occurred (479).

At very high levels of BHA intake the electrolyte balance appears to undergo a redistribution. This is evidenced by a study in which rabbits receiving daily doses of BHA at the 1,000 mg/kg level exhibited sharp increases in the urine sodium and potassium levels. Continued intake of the antioxidants resulted in muscular weakness and finally death (113).

The general low level in vivo of BHA/enzyme interactions is in sharp contrast to the recent finding that BHA shows a pronounced suppressive effect against bradykinin induced contractile response of the smooth muscle of guinea pig ileum (343, 344).

While carcinogenic activity of BHA has not been demonstrated, presumptive evidence of precancerous activity is reported for BHA applied topically to the ears of guinea pigs. What is described as microinvasion by basal cell pseudopods was found to have occurred and associated with which was the destruction of superficial connective tissue and the fragmentation of the skin collagen (352). In contrast, BHA fed to mice which had received transplanted leucosis tumors was found to be effective in prolonging the life of the experimental animals and in some instances inducing tumor recession (131).

BUTYLATED HYDROXYANISOLE

Chemical Information

I. Nomenclature (291a)

A. Common Names

Butylated hydroxyanisole

BHA

Mono butylated hydroxyanisole

B. Chemical Names

Mixture of 2-and-3-tert-butyl-4-hydroxyanisole

Mixture of 2-and-3-tert-butyl-4-methoxyphenol

C. Trade Names

Embanox

BHA

D. Chemical Abstracts Services Unique Registry Number

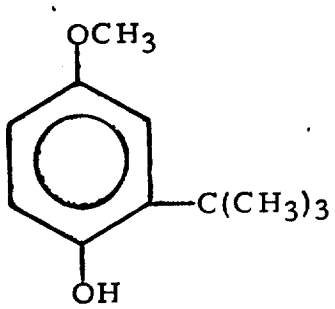
MX 8003245

II. Empirical Formula (141a)

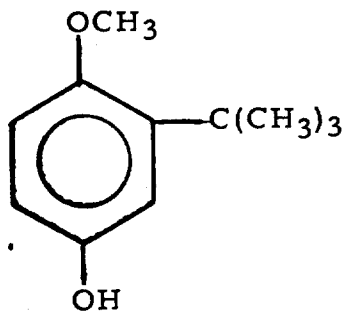
$C_{11}H_{16}O_2$

III. Structural Formulae (141a)

2-BHA



3-BHA



IV. Molecular Weight (141a)

108.25

V. Specifications

A. Chemical (141a)

The commercial material by definition is a mixture of 2-BHA and 3-BHA isomers and contains not less than 98.5% of the empirical formula $C_{11}H_{16}O_2$.

B. Food Grade (Food Chemicals Codex-First Edition) (141a)

Assay. Not less than 98.5% of $C_{11}H_{16}O_2$.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003%).

Heavy metals (as Pb). Not more than 10 parts per million (0.001%).

Residue on ignition. Not more than 0.01%.

Hydroxyanisole 0.5% maximum *

Hydroquinone 0.6% maximum *

C. Official Compendia

Food Chemicals Codex First Edition p. 91 (141a)

VI. Description

A. General Characteristics (141a)

Butylated hydroxyanisole occurs as a white or slightly yellow, waxy solid having a faint characteristic odor. It is predominately 3-tert-butyl-4-hydroxyanisole (3-BHA), with varying amounts of 2-tert-butyl-4-hydroxyanisole (2-BHA).

* Company (not identified) specifications required to be met by seller.

B. Physical Properties (Typical for Food Grade)

Dissoluble in water

Freely soluble in petroleum ether (Skellysolve H), in alcohol (>72%) and in propylene glycol.

Slightly soluble in fats and oils (<0.5%). (291a)

Melts between 48°C and 63°C. (141a)

When isomer mixture consists of approximately 15% 2-BHA and 85% 3-BHA the melting range lies between 54°C-58°C and the b. p. lies between 264°-270°C at 733 mm Hg. (174).

C. Stability in Containers.

Recommended storage in well closed containers, (141a) eg: polyethylene lined fiber drums.

VII Analytical Methods

Identification and estimation in foodstuffs and metabolites.

- Gas chromatographic (GLC) identification of BHA in BHA/BHT mixtures in breakfast cereals (423), in potato granules (437).
- TLC detection of BHA in antixodant mixtures including propyl gallate, BHT and nordihydroguaiaretic acid in fats and oils. As little as 0.5 ug of BHA can be detected (374).
- GLC detection of BHA in BHA/BHT mixtures in vegetable oils (172).
- Separation of BHA and gallates ester mixtures from fat by reversed-phase partition chromatography followed by colorimetric identification of BHA after reaction with 2,6-dichloroquinone chloroimide (51); also identification and estimation of BHA in lard (170) by this method.
- Fluorometric detection of BHA in lard, cereals, and waxed packaging liners (190).
- Colorimetric estimation of BHA in vegetables oils after isolation from carrier using solvent extraction and liquid colorimetric (206).

- GLC estimation of BHA and BHT levels in vitamin A preparations (291).

Characterization of BHA and its identification in antioxidant mixtures.

- TLC on polyamide sheets followed by color development using either Br/water - CCl_4 spray or fluorescein sodium in Me_2NOCH-EtOH (477).
- Determination of BHA isomer content using either GLC (BHA in chloroform) or NMR (60).
- Separation and identification of BHA in various gallate mixtures using TLC (103).

VIII Occurrence and levels found in:

A. Plants

(none)

B. Animals

(none)

C. Synthetics

(none)

D. Natural inorganic sources

(none)

BUTYLATED HYDROXYANISOLE

Biological Data

I. Acute Toxicity

Animal	Sex & No.	Route	Dosage mg/kg LD_0	LD_{50}	LD_{100}	Ref.
Rats	Male	Oral		2950		(101)
Rats	NA**	Oral	1000	2900	4500	(224)
Rats	NA**	Oral		2200		(262a)*
Rats	NA **	Oral		5000		(72)
Mice	NA**	Oral	1000	1250	3000	(224)
Mice	NA**	Oral		2000		(262a)*

Mixed antioxidant systems consisting of BHA and propyl gallate showed neither synergistic nor antagonistic activities at least with respect to any changes in their LD_{50} values. Thus for example a 2:1 mixture of BHA and propyl gallate resulted in an LD_{50} of 2633 mg/kg for rats while pure BHA had an LD_{50} in these animals of 2950 mg/kg and pure propyl gallate had and LD_{50} of 2500 mg/kg. (101)

* FDA 1950 unpublished data given in ref. (262a).

** Not available in abstracts.

II. Short Term Studies

In a series of sub-acute toxicity experiments with rats and mice lasting 2-1/2 months, BHA was added to the daily dietary ration in the amounts of 1/5 and 1/10 the respective acute oral LD_{50}'s (2900 mg/kg/ for the rat and 1250 mg/kg for the mouse). During this period it was observed that all the experimental animals exhibited a decrease in their growth rates and reduced activity of certain blood enzymes: catalase, peroxidase and cholinesterase (224); however pathological symptoms due to BHA were lacking. A similar low order of toxicity due to BHA was reported in a comparative study with rats and mice fed BHA or propyl gallate as part of the lipid portion of their diet. In contrast, propyl gallate did cause some pathological reactions in a number of the test animals (226).

In rats, as already noted (224), blood enzymes are somewhat affected by BHA, although most of the other blood parameters remain within normal limits. Thus in one study, rats (body weight 120-150 gms.) received daily doses of 4 mg/kg of BHA in 1 gm. of lard added to their standard diet for 30-35 days. Initially and at the 15th and 30th day of the experiment, blood sera were obtained from the animals and analyzed for dry substance, glycogen, phospholipids, fat and the iodine number. No changes in any of these indexes were found over the experimental period with the exception of the iodine number which was slightly lower for the sera of the test animals (227).

Significant increases in oxygen uptake were found to occur in liver tissue homogenates (in the presence of a succinate substrate) prepared from the

liver of white rats which had received either 0.1% or 1% BHA in their diets over an 8 week period. In addition the 1% BHA level had an effect on the phosphorylation efficiency of the hepatic mitochondria since the P/O ratio was significantly increased compared to the control group (405). Similar studies with rats fed a diet containing 0.8 gm BHA/100 gm dry food stuff over an 8 week period demonstrated that no modification occurred in the blood picture of these animals nor in the indexes of liver lipids and liver proteins. It was found, however, during this period that the leucocytes had somewhat decreased phagocytic capacity (406).

Body weight, liver weight, and the biphenyl 4-hydroxylase activity of the liver microsomes were monitored in another comparative study involving a number of different antioxidants including BHA. When fed to growing male rats over a period of 12 days it was found that BHA at 0.1 and 0.25% of the diet did not affect liver weight or the enzyme activity but at 0.5% it caused some increase in enzyme activity but not in liver weight (97).

The liver response of rats was compared following the administration of known hepatotoxins and food antioxidants by investigating liver weights, activity of liver processing enxymes, and "looking for histological changes." The antioxidants were given daily by stomach tube in graded doses up to 500mg/kg in peanut oil. After seven daily doses, growth retardation had occurred at the highest dosage level of all antioxidants except for BHA.

Hepatomegaly was noted only in those animals which had received 500 mg/kg either of coumarin, BHA or BHT. No fatty change however was observed among groups given BHA at any level (139). In this work BHA was also found to be without effect on the activity of the glucose-6-phosphatase in the liver (138).

Further studies with rats fed BHA at 0.1% level over a period of four months also showed significant increases in the urinary excretion of ascorbic acid and, as expected, increased liver weights. There was no evidence, however, of any hepatotoxic action due to the BHA, suggesting that the enlargement of the organ was rather the result of hyperfunctional activity (150). In contrast with BHT, the BHA induced hepatomegaly was not the result of increases in drug metabolizing enzymes (157, 158). However, BHA did seem to effect certain of the liver systems as determined from tissue homogenate studies. In livers removed from Wistar rats which had received 0.1% or 1% BHA in the fat of their diet for four to eight weeks before sacrifice, no significant changes were detected with regard to aldolase, glutamic-oxalacetic transaminase, alkaline and acid phosphatases, and xanthine oxidase activities. However, those receiving the higher dose over the 8 week period did show a significant increase in the liver succinate dehydrogenase activity (407).

The LD_{50} (of a 1:2 mixture of propyl gallate and BHA) in rats was 2633± 140 mg/kg. When these animals were fed a mixture of these antioxidants at 500 times the concentration permitted in the human diet (100 mg of BHA and 50 mg of propyl gallate /kg) over a period of 2-1/2 months there was no change noted in the weight gain as compared with the control animals. However, on the 20th day the blood cholinesterase and catalase decreased in the test animals [also noted by other investigators (227)]. This effect however was reversible, the activity of both the enzymes returning to normal values at the end of the experimental period. In general, greater sensitivity to these antioxidants was observed in female rats than in male rats (102). Details of BHA feeding studies on rats are presented in Tables 1 and 2.

Table 1

THE EFFECTS OF BHA IN THE DIET, OR AS ORAL DOSES, ON WEIGHT GAIN, PATHOLOGY AND REPRODUCTIVE PERFORMANCE OF RATS

Human Intake Ratio = Daily animal intake in $mgkg^{-1}$/estimated daily human intake. Estimated human intake is <0.1 mg/kg of BHA per diem (33)

Organ and Tissue abbreviations are: A = Adrenal Ao = Aorta B = Brain Bm = Bone marrow F = Fat G = GI tract Go = Gonads H = Heart K = Kidney Li = Liver Lu = Lung Ln = Lymph nodes M = Muscle P = Pancreas S = Spleen St = Stomach T = Testis Th = Thyroid UB = Urinary bladder

Level in diet or per diem dose	Duration (months)	Human Intake Ratio	No. and Sex of Animals	Wt. Gain	Gross and physiological effects	Tissues and Organs examined for pathology	Reproductive Performance and Embryotoxicity	Diet Formulation	Rfce.
0.00014%	8	1.4	15 M	Normal	Mortality, rel. organ wts. and hemoglobin levels normal	A,B,G,H,K,Li,Lu,P,S,T (Normal)		BHA added as Tenox II* in lard (1.35% of diet)	163
0.001%	8	4	40 MF	Normal	Mortality and rel. organ wts. normal	Ao,K,Li,S,T (Normal)	Performance and litter size normal	BHA added to lard; diet was 10% in lard	68
0.003%	22	30	30 MF	Normal	No gross effects	A,F,H,K,Li,Lu,M,S (Normal)	Normal, 2nd generation normal after 3 mos. feeding BHA	BHA added to lard, diet was 6% in lard	478a
0.0068%	8	68	15 M	Normal	Mortality, rel. organ wts. and hemoglobin levels normal	A,B,G,H,K,Li,Lu,P,S,T (Normal)		BHA added as Tenox II* in lard (1.35% of diet)	163
0.01%	24	100	26 MF	Normal	Mortality and rel. organ wts. normal		Performance and litter size normal	BHA added to hydrogenated coconut oil, diet was 10% in oil	68
0.06%	22	600	15 MF	Normal			Normal, 2nd generation normal after 3 mos. feeding BHA	BHA added to lard, diet was 6% in lard	478a
0.1%	8	1000	40 MF	Normal	Mortality and rel. organ wts. normal	Ao,K,Li,S,T (Normal)	Performance and litter size normal	BHA added to lard, diet was 10% in lard	68
0.1%	24	1000	40 MF	Normal	Mortality and rel. organ wts. normal	Ao,K,Li,S,T (Normal)	Performance and litter size normal	BHA added to hydrogenated coconut oil, diet was 10% in diet	68
0.12%	21	1200	17 MF	Normal		A,F,H,K,Li,Lu,S (Normal)		BHA added to lard, diet was 6% in lard	478a
0.5%	6	5000	7	Normal		A,F,H,K,Li,Lu,S (Normal)		"	
0.5%	8	5000	40 MF	Reduced	Initial wt. gain and mature wt. reduced, rel. liver wts. increased	Ao,K,Li,S,T (Normal)	Performance and litter size normal	BHA added to lard, diet was 10% in lard	68
1.0%	6	1×10^4	7	Normal		A,F,H,K,Li,Lu,S (Normal)		BHA added to lard, diet was 6% in lard	478a
2.0%	6	2×10^4	7	Reduced	Reduced wt. gain and decreased feed intake	A,F,H,K,Li,Lu,S (Normal)		"	478a
3.0%	6	3×10^4	7	Reduced	Reduced wt. gain and decreased feed intake	A,F,H,K,Li,Lu,S (Normal)		Food refusal at BHA levels of 6% and above	478a
500 mg/kg p.o	7 wks.	5000	31 F		(Dosing before mating, through pregnancy up to sacrifice prior to parturition)		Mating, fertility, no. of corpora lutea, litter size, fetal wts. normal. No abortions, no fetal abnormalities.	BHA given by intubation in arachis oil	94
750 mg/kg p.o	10 wks.	7500	7 F		(Dosing through pregnancy up to sacrifice prior to parturition)		Mating and fertility normal, no abortions, no fetal abnormalities.	"	94
1.0 g/kg p.o	(single dose)	1×10^4	21 F		(Days 9, 11 or 13 of pregnancy)				

*Composition given in Table 5.

Table 2

THE EFFECTS OF HEAT-TREATED BHA IN THE DIET ON WEIGHT GAIN, PATHOLOGY AND REPRODUCTIVE PERFORMANCE OF RATS

Human Intake Ratio is Defined and Tissue and Organ Abbreviations are Given in Table 1

Level in Diet	Duration (months)	Human Intake Ratio	No. and Sex of Animals	Wt. Gain	Gross and physiological effects	Tissues and Organs examined for pathology	Reproductive Performance	Diet Formulation	Rfnce.
0.00014%	8	1.4	104 MF	Normal	Mortality, relative organ wts. and hemoglobin levels normal	A,B, G,H,K,Li,Lu,P,S, T (Normal)	-----	BHA added as Tenox II* in lard and baked in bread	163
0.004%	12	4	40 MF	Normal	Mortality and relative organ wts. normal	Ao,K,Li,S,T (Normal)	Performance and litter size normal	BHA added to lard, then heated 30 min. at 150°. Diet 10% in lard	68
0.0068%	12	68	208 MF	Normal	Mortality and relative organ wts. normal	A,B,G,H,K,Li,Lu,P,S, (Normal)	-----	BHA added as Tenox II* in lard and baked in bread	163
0.03%	22	300	15 MF	Normal		A,F,H,K,Li,Lu,M,S, (Normal)	Performance normal, second generation normal after 3 mo. on experimental diet	BHA added to lard,heated 30 min. at 190°. Diet 6% in lard, incl. 0.1% H_3PO_4	478a
0.1%	8	1000	40 MF	Normal	Mortality and relative organ wts. normal	Ao,K,Li,S,T (Normal)	Performance and litter size normal	BHA added to lard,heated 30 min. at 150°. Diet 10% in lard	68
0.5%	8	5000	40 MF	Reduced	Initial wt. gain and mature wt. reduced	Ao,K,Li,S,T (Normal)	Performance and litter size normal	"	3

*Composition given in Table 5.

III. Long Term Studies

Despite the slow intestinal absorption of BHA reported in dogs (148), long term feeding studies with these animals were apparently without any ill consequences. Groups of three dogs each were given BHA (added to the diet as a 50% solution in propylene glycol) at various levels, viz 0, 0.3, 3.0, 30 and 100 mg/kg of body weight for one year. Periodic analysis showed no changes in urinary protein and sugar, in hematologic values or maintenance of normal growth. Necroscopy revealed no storage of BHA in fat, brain, liver and kidney and similarly no changes attributable to BHA were demonstrated (185).

In further experiments with dogs BHA was fed at levels of 0, 5, 50 and 250 mg/kg of body weight daily for 15 months. Apparently these animals can tolerate BHA levels at least 220 times the maximum allowable level (0.02%)*for this antioxidant in lard. This conclusion is supported by the general health, weight gains, hemograms and microscopy performed on the test animals. Some liver injury was found to occur in three dogs receiving the highest dosage, 250 mg/kg of body weight. As was found in earlier work, these BHA-fed dogs showed higher levels of urinary glucuronates and a higher total-to-inorganic sulfate ratio suggesting that BHA in dogs is mainly excreted by this route (479). Details of long term feeding studies on dogs are presented in Table 3.

* Code of Federal Regulations 1972 Sec. 121.101

Table 3

Table 3

THE EFFECTS OF BHA IN LONG TERM FEEDINGS TO DOGS

Human Intake Ratio is Defined and Tissue and Organ Abbreviations are given in Table 1

Level in Diet %	Duration (months)	Human Intake Ratio	No. and Sex of Animals	Wt. Gain	Gross and physiological effects	Tissues and Organs examined for pathology	Diet Formulation	Reference
0.001	12	3	3 MF	Normal	Hematology, urine protein, urine sugar and rel. organ wts. normal	A,B,Bm,G,H,K,Li,Lu,P,S, St,T,UB (Normal)	BHA added to diet in propylene glycol	185
0.01	12	30	3 MF	Normal	"	"	"	185
0.018	15	50	4 MF	Normal	Hematology normal	A,Bm,G,Go,H,Li,Ln,Lu, P, para-T (Normal)	BHA added in lard, diet was 8.0% in lard	479
0.1	12	300	3 MF	Normal	Hematology, urine protein, urine sugar and rel. organ wts. normal	A,B,Bm,G,H,K,Li,LumP S,St,T,UB (Normal)	BHA added to diet in propylene glycol	185
0.1	12	300	3 MF	Normal	"	"	"	185
0.179	15	500	4 MF	Normal	Hematology normal	A,Bm,G,Go,H,Li,Ln,Lu, P, para-T (Normal)	BHA added in lard, diet was 8% in lard	479
0.3	12	900	3 MF	Normal	Hematology, urine protein, urine sugar and rel. organ wts. normal	A,B,Bm,G,H,K,Li,Lu,P, S,St,T,UB (Normal	BHA added to diet in propylene glycol	185
0.88	15	2,500	3 MF	Reduced	Hematology normal, urine sugar elevated	A,Bm,G,Go,H,Lu,Ln,P, para-T were normal. Liver showed degenerative changes.	BHA added in lard, diet was 8% in lard	479

IV. Special Studies

One hundred and sixty male albino rats were used to study the effect of a number of different antioxidants including BHA on the activity of enterokinase and alkaline phosphatase in the small intestine mucosa as well as the secretion of amylase and lipase in the pancreas. One group of animals was kept on the diet for one month, another group for three months. With most of the antioxidants examined including BHA no changes in the enterokinase secretion was found in the mucosa of the superior portion of the small intestine and there was no change in the activity of the pancreatic lipase (228).

BHA was evaluated for possible carcinogenicity in mice. Six groups of 50 males and 50 females each were given single subcutaneous injections (10 mg/mouse) of BHA in trioctanoin during an observational period extending from 273-575 days. Another 6 groups of 50 males and 50 females each were given weekly skin applications of 0.1 mg BHA in acetone, while 6 other groups were given skin treatment with acetone solutions containing 10 mg of BHA. A third series consisting of 100 males and 100 females were treated only with acetone over an observational period extending from 310-519 days. No gross or microscopic evidence of tumor formation was detected in the skin of these mice by any route of administration. (186).

More recent work, however, finds that topical treatment with BHA results in the appearance of certain morphological lesions associated with very early stages of epidermal tumors. This condition is characterized by the protrusion of pseudopods through defective areas in the basement membrane, a phenomenon termed microinvasion. Thus when BHA, made up in concentrations

of 20% in lanolin, was applied to the ears of guinea pigs once daily for periods of 2-6 weeks, microinvasion by basal cell pseudopods occurred associated with destruction of the superficial connective tissue and fragmentation of the collagen. No such changes were observed in control guinea pigs which had either been treated with lanolin alone, or left untreated (352). On the other hand, BHA (in undisclosed amounts) fed to mice which had received transplanted leucosis tumors was found to be effective in prolonging the life of the experimental animals and in some cases introducing recession of the tumors. This demonstrated anti-tumor activity of BHA and some similar substances is explained on the basis of their ability to capture free radicals which are associated with the oxidative processes occurring apparently at high rates in these leucosis tumors (131).

Potential teratogenicity was evaluated in the offspring of two different mouse strains and four different rat strains given single or multiple doses of BHA in peanut oil. However, no teratogenic effects were observed at dose levels as high as 300-500 mg/kg administered for as long as 7 weeks although under these conditions rather high mortality rate, between 75% -85%, occurred. While a dose of 250 mg/kg of body weight resulted in a mortality rate of 33%, there was no change in the reproductive indexes for rats or for the Evans strain of mice treated with BHA. There was, however, some retardation of growth in the rats at the highest levels (500 mg/kg fed for the full 7 week period) (94).

Fetal resorption in the rat was studied in relation to a variety of antioxidants fed to pregnant animals. BHA was among those which demonstrated some beneficial effects on resorption rates, in contrast, for example, to propyl gallate among others which substantially increased the resorption rate (434).

Rats were maintained on bread containing 50 times the normal concentration of propyl gallate, ClO_2, and BHA for one year; the bread represented 75% of the diet. There was no effect on the growth or mortality of the animal nor on the organ weight or histopathology of the tissues examined. No evidence of synergistic action of the substances (162) was demonstrated. Negative results were again obtained, by these same authors, for similar study with male rats extending over a feeding period of 32 weeks (163).

Albino and Norway hooded strains of weanling rats were kept on BHA supplemented diets for 24 months and 8 months respectively; the levels of antioxidants administered ranging from 0.01% - 0.5% of the fat in the diet. At the 0.1% level of BHA, no apparent changes occurred in the parameters of growth, food consumption, reproduction, mortality, organ weights and post mortum tissue examination of the animals. Among those animals receiving 0.5% BHA no pathological changes of the spleen, kidney, liver, testes or skin were found. The ratio of heart, spleen and kidney weight, to total body weights, was no different than that for the control animals. In addition, no unusual effects were noted on one reproductive cycle of the rat. (68).

Daily administration of a mix consisting of 20 mg/kg of BHA and 10 mg/kg of propyl gallate was found to increase the mortality of male rats during a 9 month experimental period and caused their sterility. * There was no change in the blood sugar level and catalase and cholinesterase activities, but the level of ketone bodies in the blood was found to have decreased (101).

* Editor's note: This reported increase in the mortality rate may be due to the level of propyl gallate fed the test animals. A number of such adverse findings are detailed in the FDRL monograph on propyl gallate. (date: August 25, 1972, CAS REG. NO. 000121799 and ACCESSION NO. 24)

In contrast to the apparently innocuous metabolic behavior of BHA as demonstrated by the relatively low order of toxicity this antioxidant has pronounced pharmacological activity against bradykinin. Thus, the bradykinin induced contractile response elicited from the smooth muscle of guinea pig ileum is totally suppressed when BHA is present. Gallic acid and its esters also exhibit the same suppressive effect but BHA is far more active in this regard (343). It has been established that concentrations as low as 8×10^{-10} mole per liter of BHA can inhibit detectably the guinea pig's smooth muscle contraction elicited by bradykinin. There seems to be some evidence that these antioxidants might act as competitive inhibitors but the mechanism is apparently quite complex and the effect is only partially reversible (344).

BHA was also found to be a most potent ovicide against the eggs of A. albinmanus. With BHA, as well as with other aromatic and alicyclic substituted phenols, ovicidal activity is probably associated with the inhibition of tyrosine hydroxylase. This follows both from the observation that unmelanized eggs were more susceptible to the toxicants than were the melanized eggs (melanization being necessary for egg hatching), and the fact that the slowest enzymatic step in the overall synthesis of melanin involves the tyrosine hydroxylase-enzyme (294).

Rabbits fed daily doses of BHA at the 1000 mg level exhibited ten fold increase in the sodium and a 20% increase in the potassium levels of the urine, concomitant with a decrease in the volume of extracellular fluid. Continued intake of BHA at this rate resulted in a decrease in the serum potassium level which was followed by muscular weakness and finally

death. The sodium and potassium levels of the heart tissue were found to have remained constant until the onset of the terminal stages. There was also increased excretion of aldosterone and histochemically the zona glomerulosa of the adrenal cortex showed a gross decrease in fat staining (113).

When BHA was fed to rats at the level of 0.1% of the diet, there was an increase in the level of total serum cholesterol, but higher doses of the antioxidant did not produce any further elevations. (The increase in the serum cholesterol was reflected by a relatively greater increase in the amount of the serum free cholesterol than of the ester cholesterol.) In addition, increased liver weights were produced as well as increases in the sizes of the male adrenals. Yet there were none of the marked histological changes reported by other workers in the rabbit (113) when lethal doses of the antioxidant had been fed these animals. As the total liver weight increased, the absolute lipid content of liver was also increased, although there was no change in the concentration of the total and the esterified cholesterol in the liver nor on the composition of the polyunsaturated fatty acids of this organ (205).

The influence of certain organic substances added to the diet of the cotton rat was evaluated with respect to the incidence of dental caries in this species. BHA added at the 0.5% level in the diet was found to inhibit caries development some 42%, however, this large amount also was found to cause a reduction in the growth rate of the animals (435).

Biochemical Aspects

I. Breakdown

There is some evidence that in the rat the 4-butyl group of BHA does not remain intact after oral ingestion. Experiments with a labeled analogue, phenylisobutyric acid, showed that the larger of the butyl group moiety is associated with a fragment which is excreted in the urine while only a small amount of the original labeled molecule is retained in the lipid portion of the liver (54).

After single dosing with BHA (0.4 gm/kg), rats showed an increase in urinary glucuronide and in ethereal sulphate excretions over a five day period. The glucuronide and the ethereal sulphate accounted for 61-82% and 11-16% of the dose respectively while 5% was found to be excreted as unchanged BHA. When very small single oral doses of the antioxidants were given to these animals (0.002, 0.01, 0.025, 0.05, and 0.1 gm/kg) the overall recoveries from the urine ranged from 81-100% of the dose over this five day period. When 0.4 gm of the 2-isomer was fed as a single oral dose to these animals, 72% was excreted as ethereal sulphate while 57-71% of a single oral dose of the 3-isomer was found excreted as the glucuronide. A probable minor metabolite in these animals seems to be the demethylation product of the 2-isomer (33).

II. Absorption - Distribution

The kinetics of excretion in man seems to be roughly the same for BHA and BHT. In general there is an early rapid excretion in which about 50% of the intake dose appears in the urine during the first 24 hours after administration. This is followed by a slower excretion phase which probably represents the release of the compounds or their metabolites stored in tissues. Thus when ^{14}C-labeled BHA was administered to humans (male) in a single oral dose (approximately 0.5 mg/kg), the cumulative urinary excretion of radioactive material was, by the eleventh day, no more than 80%-90% of the initial dose (100).

In other studies involving man, rabbits, and rats, labeled BHA fed orally was found to be readily absorbed into the intestines and quickly excreted in the urine and feces mainly in the form of glucuronic derivatives (148). In the albino rat, for example, 90% of the BHA fed as pure tert-2-BHA isomer was excreted in the urine within 4 days (160). In dogs, on the other hand, only slight intestinal absorption occurred after oral feeding of BHA, and the material which did pass across the intestinal wall was excreted coupled with sulfuric acid (148).

The tissue storage of BHA does not appear to be a factor in the kinetics of its metabolism. Thus when 0.1% of the antioxidant was added to the diet of pigs over a period of 4 months or to the diet of pullets over an 8 week period no accumulation was found in muscle, liver, kidney or the

reserve fat of either of these species (142).

In the rabbit 1 gm of BHA fed orally resulted in the excretion of 46% of the antioxidant as glucuronides, 9% as ethereal sulphates, and 6% as free phenols. The percentage of glucuronide which appeared seems to be related to the amount of the dose originally fed the animals, thus for a 0.5 gm. dose 60% was excreted as a glucuronide, while for a 0.25 gm. dose 84% was excreted in this form. This pattern holds true only for single doses since it was found that after repeated doses of either 1 or 0.5 gm. the glucuronide percentage was lower than for any of the single doses (98).

Details of breakdown of BHA are presented in Table 4.

III. Metabolism and Excretion

Details of metabolic studies on BHA are presented in Table 4.

IV. Effects of Enzymes and Other Biochemical Parameters

No accessible data in available literature.

V. Drug Interaction

No accessible data in available literature.

VI. Consumer Exposure Information

Information presented in Table 6.

Table 4

Table 4

THE FATE OF BUTYLATED H YDROXYANISOLE IN MAMMALS

Human Intake Ratio is defined in Table 1

Species	BHA	Dose mg/kg	Route	Human Intake Ratio	No. of Doses	Urinary Excretion % in Days		Fecal Excretion % in days		Urinary Excretion Products % of dose Unchanged	Glucuronide	O-sulfate[a]	Biotransformation products characterized	Reference
Dog	Unlabelled	500	p.o meat capsule	5000	1	32	3	51	3	4	6[b]	23	O-sulfate of BHA O-sulfate of t-butylhydroquinone	34
Rabbit	Unlabelled	250	p.o	2500	1	76	1			4	60[c]	12	O-glucuronide of BHA	98
		250	p.o	2500	11	53	(17)			0	50[c]	3		
Rat	Tritiated	0.5	ip	5	1	86 91	1 4							160
	Unlabelled	2	p.o intubation	20	1	82	2-3			10	72[b]			33
	Unlabelled	100	p.o intubation	1000	1	91	7-8			4	75[b]	12	O-sulfate and O-glucuronide of BHA	33
Man	Unlabelled	0.4	p.o homogenate	4	1	36-77	1.5			<1	36-77[b]	trace		34
	^{14}C labelled in t-butyl CH_3 group	0.5	p.o capsule	5	1	60-73 80-87	2 12	0.3 on day 10						100
	Unlabelled	0.7	p.o capsule	7	1	27-71	1-2			<1	27-71[b]	trace		34

a) As increased 'ethereal' sulfate output

b) From BHA liberated by β-glucuronidase

c) As increased glucuronide output

Table 5

COMPOSITION OF TENOX® II ANTIOXIDANT

TENOX II Antioxidant:

	Percent
Butylated Hydroxyanisole (BHA)	20
Propyl Gallate	6
Citric Acid	4
Propylene Glycol	70

All percentages by weight.

COMPREHENSIVE GRAS SURVEY -- NAS/NRC 1972 10/02/72

POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	POSSIBLE DAILY INTAKE, MG. (AGE)	AVERAGE	HIGH A	HIGH B
BUTYLATED HYDROXYANISOLE NAS 0032	01 BAKED GOODS(R)	20	0-5 MO.	.053720	.071100	.063580
			6-11 MO.	.401320	.818440	.474980
			12-23 MO.	.861100	1.410240	1.019150
			2-65+ YR.	2.167760	3.220040	2.565640
BUTYLATED HYDROXYANISOLE NAS 0032	02 BREAK CERLS(R)	8	0-5 MO.	.018360	.052020	.029760
			6-11 MO.	.682380	1.829880	1.106080
			12-23 MO.	.798660	1.557540	1.294560
			2-65+ YR.	.612000	1.585080	.992000
BUTYLATED HYDROXYANISOLE NAS 0032	03 OTHER GRAIN(R)	*	0-5 MO.	.000900	.001700	.000900
			6-11 MO.	.009700	.028600	.009700
			12-23 MO.	.016400	.037900	.016400
			2-65+ YR.	.027800	.061400	.027800
BUTYLATED HYDROXYANISOLE NAS 0032	18 JAM JELLY(R)	*	0-5 MO.	**********	.000180	**********
			6-11 MO.	.004020	.013380	.011390
			12-23 MO.	.001300	.006720	.005100
			2-65+ YR.	.003420	.010620	.009690
BUTYLATED HYDROXYANISOLE NAS 0032	19 SWEET SAUCE(R)	*	0-5 MO.	.021000	.028000	.021000
			6-11 MO.	.063000	.217000	.063000
			12-23 MO.	.182000	.532000	.182000
			2-65+ YR.	.476000	1.253000	.476000
BUTYLATED HYDROXYANISOLE NAS 0032	20 GELATIN PUD(R)	10	0-5 MO.	.019900	.026730	.019900
			6-11 MO.	.126720	.384120	.126720
			12-23 MO.	.136620	.332640	.136620
			2-65+ YR.	.201960	.519750	.201960
BUTYLATED HYDROXYANISOLE NAS 0032	21 SOUPS(R)	4	0-5 MO.	.000440	.003300	.000580
			6-11 MO.	.051280	.159740	.067570
			12-23 MO.	.076560	.211420	.100920
			2-65+ YR.	.069740	.185900	.091930
BUTYLATED HYDROXYANISOLE NAS 0032	22 SNACK FOODS(R)	9	0-5 MO.	**********	.002230	**********
			6-11 MO.	.008920	.024530	.032400
			12-23 MO.	.024530	.069130	.021950
			2-65+ YR.	.028500	.082510	.108550
BUTYLATED HYDROXYANISOLE NAS 0032	23 BEV TYPE I(R)	24	0-5 MO.	.001680	.002520	.002400
			6-11 MO.	.015870	.054390	.022700
			12-23 MO.	.037940	.113750	.054200
			2-65+ YR.	.072800	.194390	.104000
BUTYLATED HYDROXYANISOLE NAS 0032	24 BEV TYPE II(R)	4	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	**********	.000080	**********
			12-23 MO.	**********	.000160	**********
			2-65+ YR.	.026000	.079520	.169000
BUTYLATED HYDROXYANISOLE NAS 0032	25 NUT PRODS(R)	11	0-5 MO.	**********	.010200	**********
			6-11 MO.	.188700	.683400	.360010
			12-23 MO.	.137700	.459000	.262710
			2-65+ YR.	.265200	.790500	.505960
BUTYLATED HYDROXYANISOLE NAS 0032	27 GRAVIES(R)	*	0-5 MO.	.000400	.001200	.000800
			6-11 MO.	.009600	.019600	.011200
			12-23 MO.	.014400	.040800	.028000
			2-65+ YR.	.033200	.085200	.066400

Table 6 (continued)

BUTYLATED HYDROXYANISOLE NAS 0032	04	FATS OILS(R)	24	0-5 MO.	.068850	.068850	.093500
				6-11 MO.	.385560	1.032750	.523600
				12-23 MO.	.867510	1.652400	1.178100
				2-65+ YR.	2.409750	4.351320	3.272500
BUTYLATED HYDROXYANISOLE NAS 0032	05	MILK PRODS(R)	*	0-5 MO.	.280260	.207600	.280260
				6-11 MO.	3.238560	15.575190	3.238560
				12-23 MO.	2.828550	9.051360	2.828550
				2-65+ YR.	2.050050	6.259140	2.050050
BUTYLATED HYDROXYANISOLE NAS 0032	06	CHEESE(R)	*	0-5 MO.	**********	.000010	**********
				6-11 MO.	.000270	.000970	.000270
				12-23 MO.	.000780	.002220	.000780
				2-65+ YR.	.000940	.002360	.000940
BUTYLATED HYDROXYANISOLE NAS 0032	07	FROZN DAIRY(R)	7	0-5 MO.	.002000	.000200	.004200
				6-11 MO.	.019000	.052800	.039900
				12-23 MO.	.028000	.067600	.000480
				2-65+ YR.	.051200	.123400	.107520
BUTYLATED HYDROXYANISOLE NAS 0032	08	PROCSD FRUT(R)	*	0-5 MO.	.028200	.075600	.037600
				6-11 MO.	.310800	.774000	.414400
				12-23 MO.	.603600	1.198200	.804800
				2-65+ YR.	.709000	1.503600	.946400
BUTYLATED HYDROXYANISOLE NAS 0032	10	MEAT PRODS(R)	9	0-5 MO.	.046200	.121800	.054560
				6-11 MO.	.869400	2.343600	1.026720
				12-23 MO.	1.268400	2.179800	1.497920
				2-65+ YR.	3.292800	5.464200	3.868640
BUTYLATED HYDROXYANISOLE NAS 0032	14	PROCSD VEGS(R)	6	0-5 MO.	.001540	.004620	.009940
				6-11 MO.	.026400	.061600	.170400
				12-23 MO.	.042900	.071830	.274900
				2-65+ YR.	.093500	.157520	.603500
BUTYLATED HYDROXYANISOLE NAS 0032	16	SOFT CANDY(R)	21	0-5 MO.	.004040	.040400	.004080
				6-11 MO.	.044440	.137360	.044880
				12-23 MO.	.070700	.187860	.071400
				2-65+ YR.	.117160	.355520	.118320
BUTYLATED HYDROXYANISOLE NAS 0032	17	CONF FROST(R)	*	0-5 MO.	**********	.001700	**********
				6-11 MO.	.001700	.003400	.001700
				12-23 MO.	.003400	.011900	.003400
				2-65+ YR.	.005100	.013600	.005100
BUTYLATED HYDROXYANISOLE NAS 0032	26	IMIT DAIRY(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	.000700	.001150	.001400
				12-23 MO.	.000400	.001700	.000800
				2-65+ YR.	.000450	.000750	.000900
BUTYLATED HYDROXYANISOLE NAS 0032	30	HARD CANDY(R)	6	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	.000040	.000120	.000180
				12-23 MO.	.000120	.000360	.000540
				2-65+ YR.	.000240	.000680	.001080
BUTYLATED HYDROXYANISOLE NAS 0032	31	CHEWING GUM(R)	4	0-5 MO.	**********	**********	**********
				6-11 MO.	.000350	.000380	.002020
				12-23 MO.	.000380	.001140	.002820
				2-65+ YR.	.000760	.001520	.005640
BUTYLATED HYDROXYANISOLE NAS 0032	48	SEAS FLAVRS(R)	*	0-5 MO.	**********	**********	**********
				6-11 MO.	**********	.010500	**********
				12-23 MO.	**********	.021000	**********
				2-65+ YR.	.010500	.052500	.010500
BUTYLATED HYDROXYANISOLE NAS 0032	ALL CATEGORIES		100	0-5 MO.	.546990	.727960	.622560
	**********			6-11 MO.	6.454780	24.223180	7.751580
	**********			12-23 MO.	8.003250	19.227270	9.918600
	**********			2-65+ YR.	12.727120	26.350020	16.330020

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